Limnol. Oceanogr., 37(1), 1992, 131–139
© 1992, by the American Society of Limnology and Oceanography, Inc.

Ammonium enhancement of dark carbon fixation and nitrogen limitation in symbiotic zooxanthellae: Effects of feeding and starvation of the sea anemone *Aiptasia pallida*

Clayton B. Cook
Bermuda Biological Station for Research, Inc., 17 Biological Station Lane, Ferry Reach GE01

Gisèle Muller-Parker
Department of Biology and Shannon Point Marine Center, Western Washington University, Bellingham 98225-9060

Christopher F. D'Elia
Chesapeake Biological Laboratory, P.O. Box 38, Solomons, Maryland 20688-0038

Abstract

The nutrient status of algae symbiotic with marine invertebrates is controversial. We assessed the nitrogen status of zooxanthellae symbiotic with the sea anemone *Aiptasia pallida* using NH_4^+ enhancement of dark C fixation; enhancement increases with N limitation in other microalgae. Freshly isolated symbionts obtained from laboratory populations of known feeding history and from field populations were assayed: NH_4^+ enhancement depended on the nutritional history of the host anemones. Zooxanthellae from well-fed laboratory populations showed little enhancement of dark ^{14}C fixation by NH_4^+, while mean dark enhancement ratios (NH_4^+ rates : seawater rates) exceeded 2.3 for zooxanthellae from anemones unfed for 1–6 weeks. Photosynthetic rates of the isolated algae declined with starvation. $V_{D'} : V_L$, an index which includes both dark NH_4^+ enhancement and photosynthesis, increased markedly with time since feeding. Three of four field samples of *A. pallida* yielded zooxanthellae with dark enhancement ratios similar to those of fed laboratory populations; $V_{D'} : V_L$ values were somewhat higher. Symbionts from anemones collected on a fourth date had a mean enhancement ratio of 2.88, and $V_{D'} : V_L$ values similar to those from laboratory anemones that had not fed for 1–2 weeks, indicative of increased N limitation.

Symbiotic dinoflagellates (zooxanthellae) are ubiquitous in oligotrophic marine environments such as coral reefs which are characterized by low levels of dissolved nutrients (D'Elia and Wiebe 1990). Most of these cells are intracellular symbionts; given this physical setting, the algae must obtain inorganic nutrients proximately from host cytoplasm. These nutrients are ultimately derived from various sources, including exogenous dissolved sources (Muscatine 1980), host catabolism (Szmant-Froelich and Pilson 1984), and host holozoic feeding (Steen 1986).

We had proposed that natural populations of symbiotic zooxanthellae may be nutrient limited (Cook and D'Elia 1987). There is evidence that zooxanthellae in sea anemones (Cook et al. 1988) and reef corals (Muscatine et al. 1989; Hoegh-Guldberg and Smith 1989) can be limited by N and possibly other nutrient elements. Such limitation would appear to be counterintuitive: cnidarian hosts (particularly sea anemones) are efficient predators on zooplankton, so that the host cell environment ought to provide a plethora of metabolites which could be utilized by algal symbionts. Indirect evidence suggests that host tissue concentrations of NH_4^+ are low (D'Elia and Cook 1988), although this has been questioned (Miller and Yellowlees 1989). As nutrient limitation of zooxanthellae and nutrient concentrations within host cells are related,

Acknowledgments

We thank Jana Orlandini for help in all phases of this work, and Ben Walter, Stephanie Reisgies, Dave Jorissen, and Ana Squellati for assistance in the lab and in the field. We are grateful to John Cullen for suggestions on the utility of ^{14}C incubations in scintillation vials, and to Alina Szmant for suggestions on the manuscript.

This study was supported by NSF BSR 84-07946 and OCE 87-18257 (C.B.C) and OCE 87-19701 (G.M.P).

Contribution 1297 of the Bermuda Biological Station for Research, Inc.

it is of considerable interest to determine the nutrient status of zooxanthellae.

In this paper we use a dark CO_2 fixation technique to examine the N status of symbiotic algae isolated from sea anemones of known nutritional history and from field populations of anemones. NH_4^+ typically stimulates dark fixation in cells with low N supply (Morris et al. 1971; Yentsch et al. 1977); the effect increases with increasing N deficiency (e.g. Goldman and Dennett 1986).

Materials and methods

Sea anemones—Two sources of the symbiotic sea anemone *Aiptasia pallida* were used in this study. A laboratory clone was used for feeding and starvation experiments. Unless otherwise stated, fed anemones were fed to excess with *Artemia* nauplii daily for a week and used the day after feeding. Starving anemones were taken from these cultures and maintained in individual Nunc tubes containing 20 ml of glass-fiber-filtered (Whatman GF/AE) low-nutrient surface water obtained from Station S in the Sargasso Sea, 12 km SE of Bermuda. This seawater was replaced weekly, and the tubes cleaned of accumulated debris. All cultures were kept in an incubator at 25°C under fluorescent illumination (12 : 12 L/D; irradiance of 80 μmol m^{-2} s^{-1}), and all experiments were conducted during the normal light cycle of the anemones.

Field anemones were obtained from Walsingham Pond, which is a mangrove pond in Bermuda. Details of nutrient levels of the pond are given by Muller-Parker et al. (1990). These anemones were collected between 0900 and 1030 hours and used within 24 h of collection.

^{14}C incubations—Suspensions of zooxanthellae were prepared by homogenizing anemones (~4–7-mm oral disc diameter) with a Teflon tissue grinder in 0.22-μm Millipore-filtered Sargasso seawater (FSSW). The cell suspensions were washed repeatedly with FSSW until a clear supernatant was obtained; final cell densities were adjusted to 3–9 $\times$ 10^5 ml^{-1} for use in experiments. Duplicate C fixation determinations were performed in 20-ml glass liquid scintillation vials (Lewis and Smith 1983). Each vial received 0.5 ml of algal suspension and FSSW with or without added NH_4Cl. Thus final cell densities ranged from 1.5 to 4.5 $\times$ 10^5 ml^{-1}. The vials were given a 20-min preincubation period in light or dark before adding 1 μCi of $NaH^{14}CO_3$ (ICN; 58 mCi $mmol^{-1}$).

We used vials wrapped with black electrical tape to measure dark C fixation. Cell suspensions in these vials were incubated in FSSW$\pm NH_4Cl$ (final concn: 20 or 50 μM). Control vials did not contain added NH_4^+. ^{14}C was added to dark vials under subdued illumination to minimize photosynthetic incorporation. Incubations with isotope were at room temperature (26°–28°C) for periods of up to 4 h. The time-course of dark C fixation was followed with bulk isolations of zooxanthellae from 5 to 6 anemones of similar nutritional history or from a single field collection. Subsequently we measured dark C fixation by zooxanthellae in 4-h incubations. Generally these were obtained from single anemones except when unfed sea anemones lacked sufficient algae; in such cases we combined symbionts from 2 to 3 anemones.

Incubations were ended by adding 0.3 ml of 6 N HCl, and unincorporated $^{14}CO_2$ was evolved on a shaking table in a hood for 20–30 min. Preliminary experiments showed that all volatile ^{14}C was removed within this time. The samples were then neutralized with an equal volume of 6 N NaOH, the tape was removed, and 10 ml of scintillation fluid (Eco-lume; ICN) were added. All samples were counted with a Packard Tri-Carb scintillation counter with automatic quench correction.

Duplicate vials of each algal suspension were incubated with ^{14}C in the light. These vials, containing 0.5 ml each of algal suspension and FSSW, were placed on a clear Plexiglas light box. The box was equipped with four 40-W "cool-white" fluorescent lamps producing a saturating irradiance of 450 μmol m^{-2} s^{-1} measured with a 2π cosine-corrected sensor. Cooling fans maintained incubation temperatures of 26°–28°C. Following the 20-min preincubation period, the vials were incubated with 1 μCi of $NaH^{14}CO_3$ for 30–40 min, then acidified and treated as above.

Each experiment included a Formalin vial

to correct for any nonvolatile ^{14}C in the stock isotope solutions. Formalin vials contained 0.5 ml of cell suspension and 0.5 ml of 20% Formalin in FSSW, buffered with saturated sodium borate. These vials were incubated and acidified with the light vials. These residual ^{14}C counts were used as background and subtracted from all other vials in a given experiment.

Specific activities were calculated from 50-μl samples of total radioactivity taken from the light and Formalin vials. The mean value of these samples was used for the specific activity for all dark vials in a given experiment. Values of ^{14}C dpm were converted to g C fixed with a standard value for the inorganic C content of surface Sargasso seawater (0.024 g liter^{-1}).

Data analysis—The effects of NH_4^+ enrichment on dark C fixation were expressed either as C fixation rates over the period of incubation (g C cell^{-1} h^{-1}) or as dark enhancement ratios. The latter were expressed either as the ratio of NH_4^+-enhanced rates to control rates, or as the ratio $V_{D'}$: V_L, where $V_{D'}$ = (dark NH_4^+ rate − dark FSSW rate), and V_L is the rate of light C fixation in FSSW (Goldman and Dennett 1986).

Time-course data were analyzed by least-squares linear regression (rates) or by paired *t*-tests comparing the amount of C fixed at each time point. Rates were determined from the slopes of plots of dark C fixation vs. time and compared by fitting confidence intervals to the slopes. The mean value of duplicate vials was used for these analyses. Comparisons of control and NH_4^+ C fixation rates in 4-h experiments (individual anemones) were made by paired *t*-tests, matching control and experimental vials containing aliquots of the same algal suspension. Multiple comparisons of groups were performed with the Tukey-Kramer posthoc ANOVA procedure: all such data satisfied Bartlett's test for homogeneity of variances.

Results

Time-course of NH_4^+ enhancement of dark C fixation—Dark uptake of inorganic C by zooxanthellae isolated from pooled individuals of *A. pallida* was generally linear for at least 4 h ($r > 0.92$; Fig. 1a–e). Cell densities in these experiments ranged from 3.5 to 4.1 $\times 10^5$ ml^{-1}. Other experiments (not shown) indicated decreasing rates after 90–120 min; these generally occurred when cell densities exceeded 4.5×10^5 ml^{-1}. In subsequent experiments we routinely used 4-h incubations to maximize differences between control (without added NH_4^+) and NH_4^+ rates and used cell densities $< 4.5 \times 10^5$ ml^{-1}.

The effect of NH_4^+ on dark C fixation in time-course experiments depended on the nutritional history of the host anemones. We used two groups of fed anemones in these experiments. One group (well fed) was fed for 5 d with saturating meals of brine shimp larvae, as indicated by the presence of uningested shimp and large boluses of egested shimp on the following morning. The second group (lightly fed) received dilute suspensions of brine shimp larvae daily for 4 d. These anemones ingested virtually all of the shimp that were presented and produced only a few small food boluses. Control rates of C uptake by symbionts from well-fed *A. pallida* were not significantly different from those with either 20 or 50 μM NH_4^+ (Fig. 1a, b; $P > 0.05$). However, the amounts of C fixed in the dark by these algae was slightly but significantly increased by the addition of both 20 μM and 50 μM NH_4^+ (Table 1; paired *t*-tests of all points in Fig. 1a, b).

The effect of NH_4^+ addition on dark C fixation was more pronounced with symbionts isolated from the lightly fed anemones (Fig. 1c, d). The overall rates of dark C fixation were increased by 52% (20 μM NH_4^+). The mean amounts of C fixed increased more than fivefold in symbionts isolated from these anemones (Table 1; $P < 0.001$ for both 20 and 50 μM).

The NH_4^+ effect was greater in symbionts isolated from anemones that had not been fed for 30 d (Fig. 1e). A 50 μM NH_4^+ spike increased the rate of C fixation over the 4-h time period by 171% compared to the control rate ($P < 0.01$; slope comparison). Figure 1e suggests that control cells from starved anemones fixed more C than control cells from fed anemones. As no such effect was seen in subsequent experiments on the effects of starvation (Fig. 2), this is probably an anomaly of this particular experiment.

Figure 1 suggested that the effects of NH_4^+ were usually not dose-dependent. Both 20

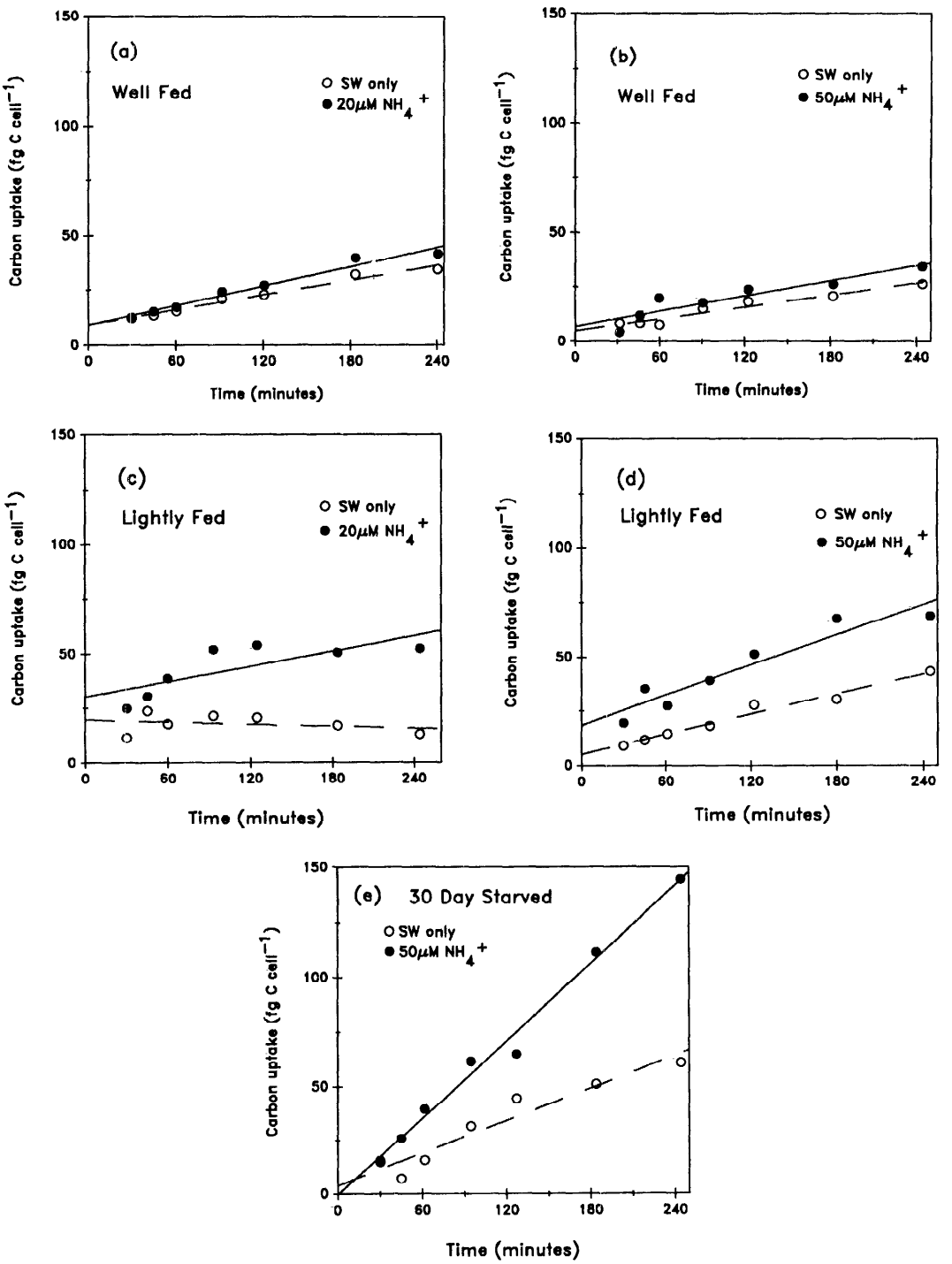

Fig. 1. Time-course of dark C fixation by zooxanthellae isolated from laboratory populations of *Aiptasia pallida*. Each point is the mean of duplicate measurements on the same cell suspension. Symbionts were obtained from pooled samples of 6–10 anemones. Least-squares linear regression lines are shown for each plot.

Table 1. Summary of *t*-test comparisons of time-course data in Figs. 1 and 3. The differences in C fixed per cell (NH_4^+ − control) were compared at each time point for each group of sea anemones. The mean values (fg C $cell^{-1}$) for all differences are given, ±1 SD. ($N = 7$ for all cases; ND—not determined.)

Source	Mean difference	
	20 μM NH_4^+	50 μM NH_4^+
Well fed	$0.35 \pm 0.30 \times 10^{-14}$ ($0.05 > P > 0.02$)	$0.47 \pm 0.49 \times 10^{-14}$ ($0.05 > P > 0.04$)
Lightly fed	$2.16 \pm 0.89 \times 10^{-14}$ ($P < 0.001$)	$2.55 \pm 1.21 \times 10^{-14}$ ($P < 0.001$)
Starved for 30 d	ND	$3.40 \pm 2.83 \times 10^{-14}$ ($0.05 > P > 0.02$)
Field	$0.44 \pm 0.66 \times 10^{-14}$ ($P > 0.05$, ns)	$0.53 \pm 0.37 \times 10^{-14}$ ($0.01 > P > 0.001$)

and 50 μM NH_4^+ tended to have similar effects, and 20 μM NH_4^+ was used in the following studies.

Effect of host starvation on NH_4^+ enhancement of dark C fixation by sea anemone symbionts—The data in Fig. 1 indicate that NH_4^+ enhancement of dark C fixation by zooxanthellae increased when sea anemones were not fed. To further investigate this, we isolated zooxanthellae from individuals of known feeding history and measured dark C fixation after 4-h incubations.

Figure 2a shows that food deprivation of *A. pallida* resulted in NH_4^+ enhancement of dark C fixation by isolated symbionts. In these experiments, 20 μM NH_4^+ did not significantly increase dark C fixation by zooxanthellae isolated from well-fed anemones (paired *t*, $P > 0.05$). The mean ratio of NH_4^+-enhanced to control dark fixation rates was 1.31 for these algae (Table 2). Zooxanthellae from anemones that were not fed for 1–6 weeks all showed significant dark enhancement by NH_4^+ in 4-h incubations ($P < 0.001$). NH_4^+ enhancement was similar for the various starvation periods; mean values for NH_4^+ : control ratios of unfed anemones ranged from 2.33 to 3.24 (Table 2). There were no differences between any of the control groups in Fig. 2a (Tukey-Kramer test; $P > 0.05$): starvation had no effect on the rate of dark C fixation in filtered seawater without added NH_4^+.

C fixation rates in the light without added NH_4^+ were also affected by the time since last feeding. Photosynthetic rates of symbionts isolated from anemones starved for 3 and 6 weeks were significantly lower than those from fed anemones and those starved for 1 week (Fig. 2b). Symbionts from the latter two groups had comparable rates of photosynthesis (Tukey multiple-range comparison; $P > 0.05$). The reduction in photosynthetic rates of algae from starving anemones may have resulted from decreased Chl *a* content (Cook et al. 1988), although we did not measure pigment content of these samples. Dark seawater rates ranged from 0.32 (fed anemones) to 0.68% (anemones starved for 6 weeks) of light rates. The $V_D : V_L$ ratio (*see methods*) combines both photosynthetic effects and NH_4^+ enhancement of dark fixation. This ratio increased with the length of time that anemones were not fed (Fig. 2c; Table 2) and seems to be a better indicator of the effects of starvation than the enhancement ratio (Table 2).

Dark C fixation by zooxanthellae from field populations of A. pallida—Figure 3 shows the effect of NH_4^+ addition on the time-course of dark C fixation by zooxanthellae from anemones obtained from Walsingham Pond, Bermuda, on 11 December 1990. The absolute rates and the effects of NH_4^+ are similar to those of the well-fed laboratory populations in Fig. 1a. The mean rates in these experiments were not affected by addition of either 20 or 50 μM NH_4^+ ($P > 0.05$). Although 20 μM NH_4^+ did not affect the amount of C fixed ($P > 0.05$) over the experiment, 50 μM NH_4^+ produced a slight increase (Table 1).

We sampled individual anemones from

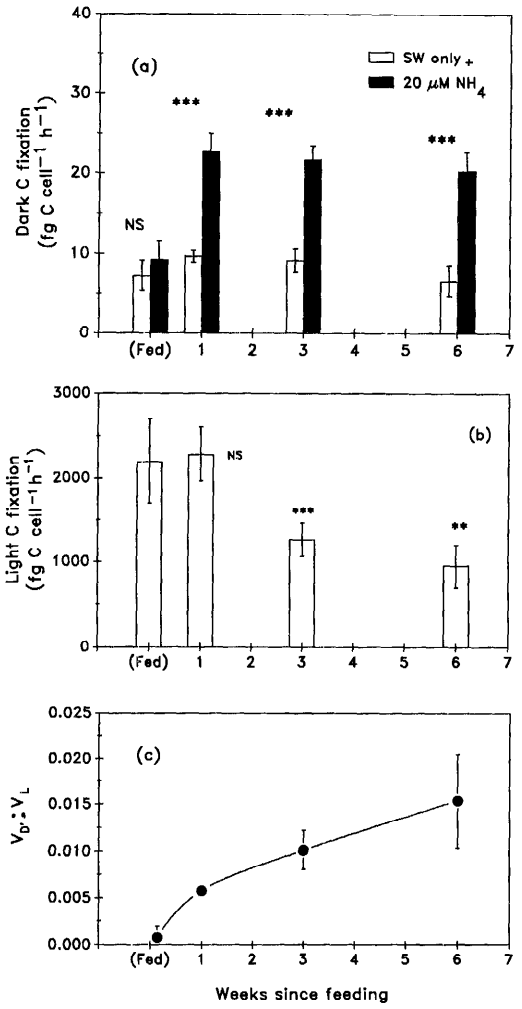

Fig. 2. Effect of host feeding on NH_4^+ enhancement and photosynthesis by zooxanthellae isolated from *Aiptasia pallida*. [a.] Dark C fixation rates of zooxanthellae assayed after 4-h incubations. Each point is the mean of values obtained from five or six individual anemones; means of duplicate determinations for each treatment used for calculations. Error bars are standard deviations. Statistical significance between controls and NH_4^+ groups was determined by paired *t*-tests of cell suspensions from each anemone. ***—$P < 0.001$. [b.] Photosynthetic rates of zooxanthellae isolated from the anemones in panel a, determined in 30-min experiments. Significance levels refer to the differences between symbionts from fed anemones and from the starved groups (Tukey multiple-comparison ANOVA). **—$P < 0.01$. [c.] $V_{D'} : V_L$ calculations of the data in panels a and b. $V_{D'} : V_L = (NH_{4\ dark} - FSSW_{dark})/FSSW_{light}$.

Walsingham Pond on four occasions in 1989 and 1990. Zooxanthellae from three of those collections had slight but significant enhancement responses of dark C fixation to 20 μM NH_4^+ (Fig. 4a). NH_4^+ : FSSW ratios for these collections were similar to those obtained from zooxanthellae of well-fed laboratory anemones, ranging from 1.30 to 1.43 (Table 2). Zooxanthellae used for the field time-course study (Fig. 3) showed a similar low level of enhancement. However, zooxanthellae from anemones obtained in a fourth collection, taken in June 1990, had much greater response to added NH_4^+ ($P < 0.001$); the mean NH_4^+ : control ratio was 2.88 (Fig. 4a; Table 2). Symbionts from all of the anemones sampled on this date showed a pronounced enhancement effect (NH_4^+ : control > 1.7) as compared to our other field samples.

$V_{D'} : V_L$ ratios for these field populations are shown in Fig. 4b. $V_{D'} : V_L$ values for field anemones other than those collected in June 1990 were about twice those of anemones fed daily in the laboratory (Fig. 2c; Table 2). $V_{D'} : V_L$ values for zooxanthellae from the June 1990 collection were at least five times higher than the other field samples and intermediate between those of laboratory anemones starved for 1 and 3 weeks (Table 2).

Discussion

The use of NH_4^+ enhancement of dark C fixation to assess the N status of zooxanthellae—NH_4^+ enhancement of dark C fixation has been widely used as an indicator of the N status of phytoplankton (Morris et al. 1971; Yentsch et al. 1977; Goldman and Dennett 1986). Dark enhancement ratios increase with N deprivation, although the $V_{D'} : V_L$ ratio is generally a more sensitive indicator of N status (Goldman and Dennett 1986). The effect results from the carboxylation of pyruvate to provide organic acids for the Krebs' cycle (Syrett 1956). In N-deficient cells, adding NH_4^+ stimulates amino acid synthesis; increased CO_2 condensation is needed to replace the acids that are used.

The technique appears to be a useful and sensitive method to assess the N status of

Table 2. Summary of NH_4^+ enhancement (NH^+: control) and $V_{D'}$: V_L ratios for the 4-h incubations shown in Figs. 2 and 4.

	Enhancement	$V_{D'}$: V_L	N
Laboratory anemones			
Feeding history			
Well fed	1.33±0.36	$0.78\pm1.2\times10^{-3}$	6
Unfed 1 week	2.35±0.14	$5.8\pm0.69\times10^{-3}$	6
Unfed 3 weeks	2.40±0.29	$10.1\pm2.05\times10^{-3}$	6
Unfed 6 weeks	3.24±0.83	$15.4\pm5.08\times10^{-3}$	5
Field anemones			
Date collected			
10 Oct 89	1.30±0.23	$1.09\pm0.46\times10^{-3}$	6
12 Dec 89	1.41±0.40	$1.42\pm0.58\times10^{-3}$	6
25 Jun 90	2.88±0.86	$7.85\pm2.07\times10^{-3}$	6
13 Dec 90	1.43±0.19	$1.66\pm0.58\times10^{-3}$	6

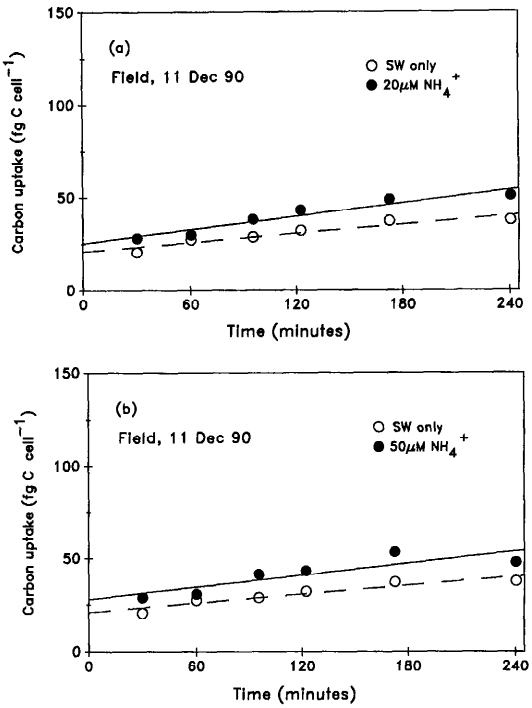

Fig. 3. Time-course of dark C fixation by zooxanthellae isolated from field populations of *Aiptasia pallida.* The anemones were collected on 11 December 1990. Symbionts were pooled from 6 anemones; linear regressions are shown.

zooxanthellae. Flynn (1990) reviewed the efficacy of this approach. The enhancement effects vary with algal species, and the technique is sensitive to incubation period and cell density. Our experiments are free of such considerations, because uptake at the cell densities used ($\leq 4.1\times10^5$ ml^{-1}) was linear for at least 4 h. Laboratory and field populations of the same host species probably are unialgal populations. Other factors that might cause problems include contamination of [^{14}C]bicarbonate stocks with nonacid volatile radioactivity and the possible loss of volatile organic acids containing ^{14}C, which would produce an underestimate of the amount of dark C fixation. As the heterotrophic synthesis of organic acids would be greatest in N-deficient cells that receive an NH_4^+ spike (Syrett 1956), this error should yield underestimates of enhancement in zooxanthellae from relatively N-poor hosts.

Host feeding and N status of zooxanthellae from A. pallida—Our results show that the NH_4^+ enhancement of dark C fixation by zooxanthellae symbiotic with *A. pallida* depends on host feeding history. Dark enhancement ratios ($+NH_4$: $-NH_4$) increase with the time that hosts are not fed in the laboratory; mean values for symbionts from anemones that had not fed for at least a week exceeded 2. Yentsch et al. (1977) had proposed this value as indicative of N limitation. Others have found that dark enhancement ratios show an "all-or-none" effect (Flynn 1990), and to some extent this is seen in our data (Table 2). As in other studies, the $V_{D'}$: V_L ratio seems to be a more sensitive indicator of N sufficiency; it shows a more consistent increase with the length of time when hosts are not fed. The time-course experiments with lightly fed anemones indicate that the amount of food ingested is also important and that both the frequency of feeding and the amount of food ingested by a host affect the results of starvation experiments. Szmant-Froelich and Pilson (1984) showed that the frequency of feeding affected NH_4^+ excretion rates in the temperate coral *Astrangia danae.* Clearly the nutritional status of zooxanthellae in our anemones—and probably other cnidarian hosts—is dependent on host feeding: food

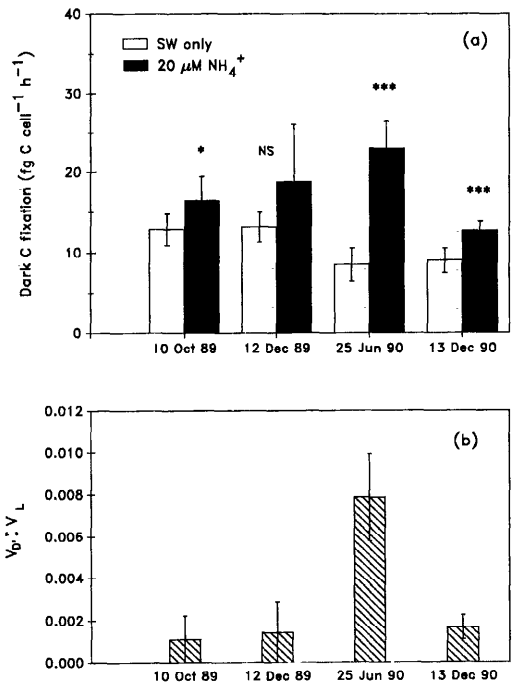

Fig. 4. Effect of 20 μM NH_4^+ addition on dark C fixation by zooxanthellae isolated from *Aiptasia pallida* collected on four dates from Walsingham Pond (4-h incubations); statistical comparisons by paired *t*-tests between treatment groups of algae from individual anemones (*see Fig. 2.*). Each value is the mean from 6 anemones. *—$P < 0.05$; ***—$P < 0.001$. [a.] Dark C fixation rates. [b.] $V_{D'} : V_L$ ratios calculated from these data and light fixation rates (not shown).

ingested by the host represents a significant source of inorganic nutrients for the algae.

The enhancement of dark C fixation by NH_4^+ in zooxanthellae from unfed *A. pallida* is consistent with our previous work with these anemones. Cessation of feeding induces nutrient deficiency in the algae, as shown by decreased growth rate, increased C:N ratios of symbionts, decreased Chl *a* content (Cook et al. 1988), and changes in nutrient fluxes. Fluxes of NH_4^+/methylamine (D'Elia and Cook 1988; Muller-Parker et al. 1988) and PO_4 (Muller-Parker et al. 1990) into intact symbiotic anemones increase with time since the last meal ingested. Such fluxes presumably reflect changes in the nutrient environment of the host cell habitat, with increasing uptake rates indicative of declining host cell concentrations. It is interesting that many of these effects, including dark enhancement, are greatest during the first 1–2 weeks after feeding stops, presumably due both to more rapid utilization of host reserves during this period and to the continual decline in the symbiont populations of these anemones (Cook et al. 1988), so that the total demand on host resources diminishes with time.

Nutrient status of zooxanthellae from field populations of A. pallida—Dark C fixation by zooxanthellae from field populations of *A. pallida* in Walsingham Pond indicated that these zooxanthellae are usually quite nutrient sufficient. The time-course data and dark enhancement ratios of symbionts from the October and December collections were similar to those of algae from well-fed hosts in the laboratory, although $V_{D'} : V_L$ data suggest that the field populations are slightly more N deficient. Analyses of PO_4 fluxes (Muller-Parker et al. 1990) support these observations. Walsingham Pond is an enclosed mangrove pond that receives a significant input of N and P from mangrove detritus and groundwater. Dissolved nutrient levels are generally higher than those of typical reef and other inshore environments in Bermuda (Muller-Parker et al. 1990). Zooplankton concentrations are generally higher than elsewhere in Bermuda and include a population of mysids that can be abundant at times (C. B. Cook pers. obs.). Thus, through most of the year there should be a ready source of particulate food for the anemones and nutrient sources for the algae.

However, our June sampling produced anemones containing zooxanthellae with apparently severe nutrient limitation. This observation suggests seasonal variation in the nutrient status, and presumably nutrient supply, of these algae. There may be transient declines in food or nutrient availability that could account for the N-deficient samples that were found. Such variation in plankton availability might account for the variation in dark enhancement patterns in zooxanthellae from these anemones (Fig. 4).

In conclusion, the NH_4^+ enhancement technique is a useful approach to assess the N status of both laboratory and field populations of symbiotic dinoflagellates. It has the same utility for these algae as with other

microalgae and yields comparable results. As in previous studies, the physiology of freshly isolated symbionts appears to reflect the nutritional status of intact symbioses (D'Elia et al. 1983; D'Elia and Cook 1988; Muller-Parker et al. 1988).

References

COOK, C. B., AND C. F. D'ELIA. 1987. Are natural populations of zooxanthellae ever nutrient-limited? Symbiosis **4:** 199–212.

———, ———, AND G. MULLER-PARKER. 1988. Host feeding and nutrient sufficiency for zooxanthellae in the sea anemone *Aiptasia pallida.* Mar. Biol. **98:** 253–262.

D'ELIA, C. F., AND C. B. COOK. 1988. Methylamine uptake by zooxanthellae/invertebrate symbioses: Insights into host ammonium environment and nutrition. Limnol. Oceanogr. **33:** 1153–1165.

———, S. L. DOMOTOR, AND K. L. WEBB. 1983. Nutrient uptake kinetics of freshly isolated zooxanthellae. Mar. Biol. **75:** 157–167.

———, AND W. J. WIEBE. 1990. Biogeochemical cycles in coral-reef ecosystems, p. 49–74. *In* Z. Dubinsky [ed.], Coral reefs. Elsevier.

FLYNN, K. J. 1990. The determination of nitrogen status in microalgae. Mar. Ecol. Prog. Ser. **61:** 297–307.

GOLDMAN, J. C., AND M. R. DENNETT. 1986. Dark CO_2 uptake by the diatom *Chaetoceros simplex* in response to nitrogen pulsing. Mar. Biol. **90:** 493–500.

HOEGH-GULDBERG, O., AND G. J. SMITH. 1989. The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. J. Exp. Mar. Biol. Ecol. **129:** 279–303.

LEWIS, M. R., AND J. C. SMITH. 1983. A small volume, short-incubation-time method for measurement of photosynthesis as a function of incident irradiance. Mar. Ecol. Prog. Ser. **13:** 99–102.

MILLER, D. J., AND D. YELLOWLEES. 1989. Inorganic nitrogen uptake by symbiotic marine cnidarians: A critical review. Proc. R. Soc. Lond. Ser. B **237:** 109–125.

MORRIS, I., C. S. YENTSCH, AND C. M. YENTSCH. 1971. The physiological state with respect to nitrogen of phytoplankton from low-nutrient subtropical water as measured by the effect of ammonium ion on dark carbon dioxide fixation. Limnol. Oceanogr. **16:** 859–868.

MULLER-PARKER, G., C. B. COOK, AND C. F. D'ELIA. 1990. Feeding affects phosphate fluxes in the sea anemone *Aiptasia pallida.* Mar. Ecol. Prog. Ser. **60:** 283–290.

———, C. F. D'ELIA, AND C. B. COOK. 1988. Nutrient limitation of zooxanthellae: Effects of host feeding history on nutrient uptake by isolated algae. Proc. 6th Int. Coral Reef Symp. **3:** 15–19.

MUSCATINE, L. 1980. Uptake, retention, and release of dissolved inorganic nutrients by marine alga-invertebrate associations, p. 229–244. *In* C. B. Cook et al. [eds.], Cellular interactions in symbiosis and parasitism. Ohio State.

———, P. G. FALKOWSKI, Z. DUBINSKY, P. A. COOK, AND L. R. MCCLOSKEY. 1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. R. Soc. Lond. Ser. B **236:** 311–324.

STEEN, R. G. 1986. Evidence for heterotrophy by zooxanthellae in symbiosis with *Aiptasia pulchella.* Biol. Bull. **170:** 267–278.

SYRETT, P. J. 1956. The assimilation of ammonia and nitrate by nitrogen-starved cells of *Chorella vulgaris.* 4. The dark fixation of carbon dioxide. Physiol. Plant. **9:** 165–171.

SZMANT-FROELICH, A., AND M. E. Q. PILSON. 1984. Effects of feeding frequency and symbiosis with zooxanthellae on nitrogen metabolism and respiration of the coral *Astrangia danae.* Mar. Biol. **81:** 153–162.

YENTSCH, C. M., C. S. YENTSCH, AND L. R. STRUBE. 1977. Variations in ammonium enhancement, an indication of nitrogen deficiency in New England coastal phytoplankton populations. J. Mar. Res. **35:** 537–555.

Submitted: 29 January 1991
Accepted: 6 August 1991
Revised: 28 August 1991